



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF "JIN BATU" (STROBILANTHES CRISPUS) EXTRACT
ON SERUM LIPID PROFILE AND ANTIOXIDANT STATUS OF
HYPERCHOLESTEROLEMIA-INDUCED RABBITS**

NURHAFZAN ANIS BINTI ISMAIL

FPSK(M) 2007 4



30 MAY 2008

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By

NURHAFZAN ANIS BINTI ISMAIL

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

February 2007



To my beloved husband, mummy and papa with love.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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NURHAFZAN ANIS BINTI ISMAIL

February 2007

Chairman: Professor Maznah Ismail, PhD

Faculty : Medicine and Health Sciences

Hypocholesterolemic, anti-atherogenic, toxicity effects and changes in the antioxidant status of *Strobilanthes crispus* extract (SCE) in atherogenically induced animal model were studied. As much as, $58.05 \pm 1.08\%$ total dietary fiber (TDF), $54.61 \pm 3.92\%$ insoluble dietary fiber (IDF) and $6.01 \pm 0.82\%$ soluble dietary fibers (SDF) was found in the *Strobilanthes crispus* (SC) ground, dried leaves (AOAC method). At concentration of 0.32-5.12 mg/ml, SCE showed $3.76 \pm 7.45\%$ to $56.72 \pm 2.49\%$ scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals with EC_{50} of 2.21 mg/ml. The total flavonoids content in SC fresh leaves and ground, dried leaves were $0.99 \pm 0.04 \%$ and $0.46 \pm 0.06 \%$, respectively. Quercetin was found highest in SC ground, dried leaves, followed by kaempferol, luteolin and rutin while kaempferol was found highest in SCE, followed by quercetin, luteolin and rutin (HPLC method). Twenty-four adult female New Zealand white rabbits (1.8-2.5 kg) were randomly assigned into four groups ($n=6/\text{group}$) and fed with normal diet; negative control (NC),

0.25% high cholesterol diet (HCD); positive control (PC), 0.25% HCD + SCE (0.2% of diet); SC and 0.25% HCD + simvastatin (SV) (20 mg/kg body weight); SV for 12 weeks. Food and water were given *ad libitum*. Blood samples were biweekly drawn for serum lipid profiles, alanine amino transferase (ALT), gamma glutamic transpeptidase (GGT), urea, creatinine, conjugated diene (CD), malondialdehyde (MDA) levels of serum, liver, kidney, heart and brains. After being killed, ascending aorta, liver, kidney and heart tissue specimens were excised immediately and prepared for the histopathological studies. PC group showed significantly increased ($p < 0.05$) TC, LDL and HDL levels, HDL/TC ratio, LDL/HDL ratio, atherogenic index, CD levels, serum, heart, liver and kidney MDA levels, and relative liver weight. Significantly ($p < 0.05$) elevated HDL and reduced kidney MDA levels were observed in SC group, while significantly reduced ($p < 0.05$) TC, LDL, heart, liver and serum MDA levels found in SV group. Massive macrophages, foam cells and atheroma plaque formation were detected, which slightly increased intima to media ratio and thickened the PC group's aorta. Moderate to fairly intense lymphocyte infiltration, slight macrovesicular lipid droplets, edematous hepatocytes, inconsistent binucleated cells and vacuolated cytoplasm were found in PC group's liver. Significantly prevented ($p < 0.05$) atheroma plaque formation, both treatment groups showed lesser aortas' thickening, irregular mild edematous hepatocytes, binucleated cells, vacuolated cytoplasm and lipid droplets inhibition. Though mild lymphocyte infiltration was found in SV, such changes were almost invisible in SC group. Briefly, SC plant may possess the hypocholesterolemic, anti-atherogenic and hepatoprotective effect due to the antioxidative properties of flavonoids, i.e. quercetin, kaempferol, luteolin, rutin and catechin.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN EKSTRAK JIN BATU (*STROBILANTHES CRISPUS*) TERHADAP
PROFIL LIPID DAN STATUS ANTIOKSIDAN SERUM ARNAB TERARUH
HIPERKOLESTEROLEMIA**

Oleh

NURHAFZAN ANIS BINTI ISMAIL

Februari 2007

Pengerusi: Profesor Maznah Ismail, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kesan hipokolesterolemik, anti-aterogenik, ketoksikan dan perubahan status antioksidan ekstrak *Strobilanthes crispus* (SCE) terhadap model haiwan diaruh aterogenik dikaji. Sebanyak $58.05 \pm 1.08\%$ serat diet jumlah (TDF), $54.61 \pm 3.92\%$ serat diet tak larut (IDF) dan $6.01 \pm 0.82\%$ serat diet larut (SDF) telah diperolehi di dalam daun kering SC terkisar (kaedah AOAC). Pada kepekatan 0.32-5.12 mg/ml, SCE telah menunjukkan sebanyak $3.76 \pm 7.45\%$ hingga $56.72 \pm 2.49\%$ kesan perencatan terhadap radikal 1,1-difenil-2-pikrilhidrazil (DPPH) dengan $EC_{50}=2.21$ mg/ml. Jumlah kandungan flavonoid yang terdapat di dalam daun *Strobilanthes crispus* (SC) segar dan kering terkisar ialah masing-masing sebanyak $0.99 \pm 0.04\%$ dan $0.46 \pm 0.06\%$. Kuersetin didapati tertinggi di dalam daun kering SC terkisar, diikuti oleh kemferol, luteolin dan rutin, manakala kemferol didapati tertinggi di dalam ekstrak kasar SC diikuti oleh kuersetin, luteolin dan rutin (kaedah HPLC). Sebanyak 24 ekor arnab putih betina dewasa New Zealand (1.8-2.5 kg) telah dibahagikan secara rawak kepada empat kumpulan ($n=6$ /kumpulan); dan

diberikan diet normal; kawalan negatif (NC), 0.25% diet berkolesterol tinggi (HCD); kawalan positif (PC), 0.25% HCD + SCE (0.2% daripada diet); SC dan 0.25% HCD + simvastatin (20 mg/kg berat badan); SV, selama 12 minggu. Arnab-arnab tersebut diberi makanan dan minuman tanpa halangan. Sampel darah diambil pada setiap dua minggu bagi analisis profil lipid, alanin aminotransferase (ALT), gamma glutamil transpeptidase (GGT), urea, kreatinin, diene terkonjugat (CD) serum dan malondialdehid (MDA) di dalam serum, hepar, ginjal, jantung dan otak. Setelah dibunuh, spesimen tisu aorta, hepar, ginjal dan jantung diambil dengan segera dan disediakan bagi kajian histopatologi. Kumpulan PC menunjukkan peningkatan kolesterol jumlah (TC), lipoprotein berketumpatan rendah (LDL) dan nisbah lipoprotein berketumpatan tinggi kepada kolesterol jumlah (HTR), nisbah LDL/HDL, indeks aterogenik, kepekatan MDA serum, jantung, hepar dan ginjal, kepekatan CD serum serta berat hepar relatif yang signifikan ($p < 0.05$). Peningkatan paras lipoprotein berketumpatan tinggi (HDL) dan juga pengurangan paras MDA ginjal secara signifikan ($p < 0.05$) telah didapati oleh kumpulan SC. Manakala kumpulan SV menunjukkan kesan penurunan paras TC, LDL, MDA jantung, hepar dan serum secara signifikan ($p < 0.05$). Sejumlah besar makrofaj, sel buih dan lesi aterosklerosis telah dikesan, yang meningkatkan nisbah intima terhadap media dan menebalkan aorta kumpulan PC. Penyerapan limfosit dari yang sederhana ke agak padat, titisan kecil lipid makrovesikular, hepatosit beredema, kehadiran sel dengan dua nukleus dan sitoplasma bervakuol yang tidak konsisten dilihat pada kumpulan PC. Dengan percncatan pembentukan plak ateroma yang signifikan ($p < 0.05$), kedua-dua kumpulan rawatan menunjukkan aorta yang kurang menebal, sedikit hepatosit beredema, sel dengan dua nukleus dan sitoplasma bervakuol yang tidak

konsisten dan perencanaan titisan lipid. Walaupun penyerapan limfosit yang rendah telah dikesan di dalam kumpulan SV, perubahan tersebut hampir tidak kelihatan di dalam kumpulan SC. Kesimpulannya, tumbuhan SC mungkin memiliki ciri-ciri hipokolesterolemik, anti-aterogenik dan perlindungan hepar disebabkan ciri-ciri antioksidatif flavonoid, contohnya kuersetin, kemferol, luteolin dan rutin serta katekin.

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I certify that an Examination Committee has met on 15th February, 2007 to conduct the final examination of Nurhafzan Anis binti Ismail on her Master of Science thesis entitled "Effects of "Jin Batu" (*Strobilanthes crispus*) Extract on Serum Lipid Profile and Antioxidant Status of Hypercholesterolemia-Induced Rabbits" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



NURHAFZAN ANIS BINTI ISMAIL

Date: 27 APRIL 2007

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
Apo	Apolipoprotein
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
BHT	Butylated hydroxyl toluene
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
CD	Conjugated Diene
GGT	Gamma Glutamyltranspeptidase
H&E	Hematoxylin and Eosin
HDL	High Density Lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl Coenzyme A
HPLC	High Performance Liquid Chromatography
HTR	High Density Lipoprotein to Total Cholesterol Ratio
IDF	Insoluble Dietary Fiber
LDL	Low Density Lipoprotein
LM	Light Microscopy
MDA	Malondialdehyde
NC	Negative Control
PC	Positive Control
SDF	Soluble Dietary Fiber

SV	Simvastatin
SC	<i>Strobilanthes crispus</i>
TAS	Total Antioxidant Status
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TC	Total Cholesterol
TDF	Total Dietary Fiber
TG	Triglycerides
VLDL	Very Low Density Lipoprotein

CHAPTER 1

INTRODUCTION

1.1 Research Background

The World Health Organization (WHO) attributes 12 million deaths a year worldwide to cardiovascular diseases (CVD). The disease involves disorders of the blood circulation system and pathological changes in blood vessels associated mainly with the heart and brain (Cruetz, 2005). Among the specific cardiovascular disease, ischaemic heart disease (IHD), often called coronary heart disease (CHD), and ischaemic stroke (insufficient blood flow to a region of the brain) accounted for the main causes of mortality.

According to a study by Khoo *et al.* (1991), in 1950 CVD was the third biggest killer in Malaysia. Twenty years later, the disease has emerged as the number one killer in Malaysia. In this new millennium era, CVD remains as prevalent as ever. The disease has been reported as the most deadly disease in developing countries (WHO Monica Project, 1988). The dietary pattern in these countries have become westernized after rapid growth of their economies. For instance, according to a data from the Nutrition Society of Malaysia, in 1961 Malaysians consumed 70% vegetables and 30% animal products. However, in 1997 Malaysians were eating 45% vegetables and 55% animal products. There has been an increase in the consumption of refined carbohydrates such as simple sugars in coffee, cookies, cakes and increase in fats in the diet, especially

saturated fats from animal sources. As a result, the number of people suffering from diet-related diseases such as obesity, diabetes, hypertension, CVD and various cancers has increased significantly (Krauss *et al.*, 1998; Kritchevsky, 1995; Sabaratnam, 2003).

Atherosclerosis is the underlying disorder in the majority of patients with CVD. Atherosclerosis refers to the build up of fatty material in the arterial wall, which leads to narrowing of an artery with potential blockage. Elevated levels of plasma or serum low density lipoprotein (LDL) cholesterol is associated with atherosclerosis (Linder, 1991; Leys *et al.*, 2002). It is now well established that statins (also known as HMG-CoA Reductase inhibitors) are potent and effective but expensive drugs for treating hypercholesterolemia. According to the Health Ministry of Malaysia (MOH), expenditure to treat heart diseases increased from RM 226 millions in 1996 to RM 751 millions in 2003. The use of statins in the primary prevention of atherosclerosis alone cost nearly RM 10 millions annually (Cruetz, 2005). Apart from this, subsidy payments for National Heart Institute (IJN) to treat civil servants and poor patients rose from RM 31.3 million between September 1992 and August 1993 to RM 144.5 million between September 2003 and August 2004. These statistics reflect the financial burden borne by the Government due to CVD.

Coronary heart diseases increased from 27% of total cardiovascular deaths in 1985 to 30.5% in 2002 (Cruetz, 2005). In 2003, cardiovascular diseases accounted for 120,295 admissions in government hospital or 7% of total admissions. From this, 5,162 were terminal cases representing 14.2% of all terminal cases (MOH, 2003).

Recently, the trend of going back to nature is getting popular in Malaysia and globally. Demand for natural health supplements (e.g.: vitamins and herbal remedies) is increasing since modern medicine and synthetic drugs have not been totally successful in solving health problems such as cancer and heart disease. Despite the beneficial LDL cholesterol lowering potential of statins, liver function test monitoring is required with the statins treatment since statins is related with possible hepatic dysfunction, myopathy together with muscle pain, tenderness or weakness. Therefore, the growing public alarm about the hazards associated with excessive use of synthetic drugs has revived the interest in the use of herbal medicines.

More Malaysians are taking active measures off illnesses and maintain good health by taking supplements. There has also been an increasing worldwide recognition of the important role of traditional herbal medicine. For example, the WHO promotes the use of herbal medicines for certain conditions such as arthritis, asthma, diabetes, stroke and vaginitis (Natila, 2002). Locally, the Forest Research Institute of Malaysia (FRIM), together with a private company is helping to upgrade the traditional medicine industry and preserve trees and herbal plants with medicinal values.

The current global trend shows that herbal therapy enters the mainstream medicine as being observed from 1970 to 2000 (Wazir, 2003). Herbs and medicinal plants are mainly used for flavours and fragrance, biopesticides, pharmaceutical and nutraceuticals. Most of Malaysian pharmaceutical products are mainly analgesics, antacids, diuretics, antibiotics and anti-histamines in the form of tablets, capsules, drops,